

CLAIMS

What is claimed is:

- 5 1. A method of determining whether a sequence of a first portion of a polynucleotide of a first organism and a sequence of a first portion of a polynucleotide of a second organism comprise a mismatch, the method comprising:
 - 10 preparing a plurality of duplexes, the duplexes comprising:
 - (i) a first polynucleotide strand having a sequence corresponding to a sequence of a first portion of genomic DNA of a first organism; and
 - (ii) a first complementary polynucleotide strand having a sequence corresponding to a sequence of a first portion of genomic DNA of a second organism;
 - 15 subjecting the duplexes to temperature gradient electrophoresis to obtain first electrophoresis data indicative of the presence of a mismatch between (a) the sequence of the first portion of the genomic DNA of the first organism and (b) the sequence of the complementary portion of the genomic DNA of the second organism.
- 20 2. The method of claim 1, comprising:
 - preparing a plurality of different duplexes, the duplexes comprising:
 - (i) a first polynucleotide strand having a sequence corresponding to a sequence of one of a plurality of different portions of the genomic DNA of the first organism; and
 - (ii) a first complementary polynucleotide strand having a sequence corresponding to a sequence of one of a plurality of different portions the genomic DNA of the second organism;
 - 25 subjecting the plurality of different duplexes to temperature gradient electrophoresis to obtain, for each of the different duplexes, first electrophoresis data indicative of the presence of a mismatch between (a) the sequence of the first portion of the genomic DNA of the first organism and (b)

the sequence of the complementary portion of the genomic DNA of the second organism.

3. A method of determining whether a sequence of a first portion of a polynucleotide of a first organism and a sequence of a first portion of a 5 polynucleotide of a second organism comprise a difference, the method comprising:

amplifying at least a first portion of a polynucleotide of a first organism to prepare amplicons of the first organism, the amplicons of the first organism corresponding to a sequence of the polynucleotide of the first 10 portion of the first organism;

amplifying at least a first portion of a polynucleotide of a second organism to prepare amplicons of the second organism, the amplicons of the second organism corresponding to a sequence of the polynucleotide of the first portion of the second organism;

15 preparing a plurality of duplexes, at least some of the duplexes comprising amplicons of the first organism and amplicons of the second organism;

20 subjecting the duplexes to temperature gradient electrophoresis to obtain first electrophoresis data indicative of the presence of a difference between (a) a sequence of the first portion of the polynucleotide of the first organism and (b) a sequence of the first portion of the polynucleotide of the second organism.

4. The method of claim 3, comprising determining the presence of a difference between the sequence of the first portion of the polynucleotide of the first organism and the sequence of the first portion of the polynucleotide of the second organism based on the electrophoresis data.

25 5. The method of claim 4, wherein the sequence of the first amplicon of the second organism is known and the method comprises determining a sequence of the first portion of the polynucleotide of the first organism based 30 on the electrophoresis data and the known sequence of the first amplicon of the second organism.

6. The method of claim 3, wherein the first organism is a first mammal.
 7. The method of claim 6, wherein the first organism is a human.
 8. The method of claim 7, wherein the second organism is a second, different mammal.
- 5 9. The method of claim 8, wherein the second organism is a second, different human.
10. The method of claim 10, wherein the polynucleotide of the first organism comprises genomic DNA of the first organism.
11. The method of claim 10, wherein the polynucleotide of the second organism comprises genomic DNA of the second organism.
12. The method of claim 11, wherein the first amplicons of the first organism and the first amplicons of the second organism are prepared concomitantly.
13. The method of claim 12, wherein the first amplicons of the first organism and the first amplicons of the second organism are prepared by a multiplexed amplification reaction.
14. The method of claim 11, wherein the method further comprises:
amplifying at least a second portion of the polynucleotide of the first organism to prepare second amplicons of the first organism, the second amplicons of the first organism corresponding to a sequence of the second portion of the polynucleotide of the first organism;
- 20 amplifying at least a second portion of the polynucleotide of the second organism to prepare second amplicons of the second organism, the second amplicons of the second organism corresponding to a sequence of the second portion of the polynucleotide of the second organism;
- 25 preparing a plurality of second duplexes, at least some of the duplexes comprising second amplicons of the first organism and second amplicons of the second organism;
- subjecting the second duplexes to temperature gradient electrophoresis to obtain first electrophoresis data indicative of the presence of

a difference between (a) a sequence of the second portion of the polynucleotide of the first organism and (b) a sequence of the second portion of the polynucleotide of the second organism.

15. A method of determining whether a sequence of a first portion of a
5 polynucleotide of a first organism and a sequence of a first portion of a polynucleotide of a second organism comprise a difference, the method comprising:

amplifying at least a first portion of a polynucleotide of a first organism to prepare first amplicons of the first organism;

10 amplifying at least a first portion of a polynucleotide of a second organism to prepare first amplicons of the second organism;

denaturing the first amplicons of the first organism;

denaturing the first amplicons of the second organism;

subjecting a mixture comprising the respective denatured first

15 amplicons of the first and second organisms to annealing; and

subjecting the respective first amplicons of the first and second organisms to temperature gradient electrophoresis to obtain first electrophoresis data indicative of the presence of a difference between (a) a sequence of the first portion of the polynucleotide of the first organism and (b)
20 a sequence of the first portion of the polynucleotide of the second organism.

16. The method of claim 15, comprising determining the presence of a difference between the sequence of the first portion of the polynucleotide of the first organism and the sequence of the first portion of the polynucleotide of the second organism based on the electrophoresis data.

25 17. The method of claim 16, wherein the sequence of the first amplicon of the second organism is known and the method comprises determining a sequence of the first portion of the polynucleotide of the first organism based on the electrophoresis data and the known sequence of the first amplicon of the second organism.

30 18. The method of claim 15, wherein the first organism is a first mammal.

19. The method of claim 18, wherein the first organism is a human.

20. The method of claim 19, wherein the second organism is a second, different mammal.
21. The method of claim 20, wherein the second organism is a second, different human.
- 5 22. The method of claim 21, wherein the polynucleotide of the first organism comprises genomic DNA of the first organism.
23. The method of claim 22, wherein the polynucleotide of the second organism comprises genomic DNA of the second organism.
- 10 24. The method of claim 23, wherein the first amplicons of the first organism and the first amplicons of the second organism are prepared concomitantly.
25. The method of claim 24, wherein the first amplicons of the first organism and the first amplicons of the second organism are prepared by a multiplexed amplification reaction.
- 15 26. The method of claim 23, wherein the method further comprises:
amplifying at least a second portion of the polynucleotide of the first organism to prepare second amplicons of the first organism;
amplifying at least a second portion of the polynucleotide of the second organism to prepare second amplicons of the second organism;
20 denaturing the second amplicons of the first organism;
denaturing the second amplicons of the second organism;
subjecting a mixture comprising the respective denatured second amplicons of the first and second organisms to annealing.
27. The method of claim 26, wherein the mixture comprising the respective denatured first amplicons of the first and second organisms and the mixture comprising the respective denatured second amplicons of the first and second organisms are the same mixture.
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30 28. The method of claim 26, wherein the step of subjecting the respective first amplicons of the first and second organisms to temperature gradient electrophoresis comprises subjecting the respective second amplicons of the

first and second organisms to temperature gradient electrophoresis to obtain
second electrophoresis data indicative of the presence of a difference between
a sequence of the second portion of the polynucleotide of the first organism
and a sequence of the second portion of the polynucleotide of the second
organism.

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29. The method of claim 28, comprising determining the presence of a difference between the sequence of the second portion of the polynucleotide of the first organism and the sequence of the second portion of the polynucleotide of the second organism based on the electrophoresis data.
- 10 30. The method of claim 28, wherein the first and second amplicons of the first and second organisms are prepared concomitantly.
31. The method of claim 30, wherein the first and second amplicons of the first and second organisms are prepared by a multiplexed amplification reaction.
- 15 32. The method of claim 28, wherein the step of subjecting comprises jointly subjecting the first and second amplicons of the first and second organisms to temperature gradient electrophoresis along a common electrophoresis lane.
33. The method of claim 32, wherein the first amplicons of the first and 20 second organisms and the second amplicons of the first and second organisms exhibit different electrophoretic migration velocities, the electrophoretic migration velocities of the first and second amplicons differing by an amount sufficient to determine the presence of one of the first and second amplicons in the presence of the other of the first and second amplicons even in the absence of (a) a difference between the sequence of the first portion of the polynucleotide of the first organism and the sequence of the first portion of the polynucleotide of the second organism and (b) a difference between the sequence of the second portion of the polynucleotide of the second organism and the sequence of the second portion of the polynucleotide of the second organism.
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34. The method of claim 32, wherein the first and second amplicons each comprise a length of at least 50 bp.
35. The method of claim 34, wherein the first and second amplicons each comprise a length of at least 150 bp.
- 5 36. The method of claim 34, wherein the first and second amplicons have different lengths.
37. The method of claim 32, wherein the electrophoresis lane is a capillary.
38. The method of claim 26, wherein the method further comprises:
10 amplifying at least a third portion of the polynucleotide of the first organism to prepare third amplicons of the first organism;
amplifying at least a third portion of the polynucleotide of the second organism to prepare third amplicons of the second organism;
denaturing the third amplicons of the first organism;
15 denaturing the third amplicons of the second organism;
subjecting a mixture comprising the respective denatured third amplicons of the first and second organisms to annealing.
39. The method of claim 38, wherein the mixture comprising the respective denatured first amplicons of the first and second organisms, the
20 mixture comprising the respective denatured second amplicons of the first and second organisms are the same mixture, and the mixture comprising the respective denatured third amplicons of the first and second organisms are the same mixture.
40. The method of claim 38, wherein the first, second, and third amplicons
25 of the first and second organisms are prepared concomitantly.
41. The method of claim 40, wherein the first, second, and third amplicons of the first and second organisms are prepared by a multiplexed amplification reaction.

42. The method of claim 38, wherein the step of subjecting the respective first amplicons of the first and second organisms to temperature gradient electrophoresis comprises subjecting the second and third amplicons of the first and second organisms to temperature gradient electrophoresis to obtain
- 5 (a) second electrophoresis data indicative of the presence of a difference between a sequence of the second portion of the polynucleotide of the first organism and a sequence of the second portion of the polynucleotide of the second organism and (b) third electrophoresis data indicative of the presence of a difference between a sequence of the third portion of the polynucleotide of the first organism and a sequence of the third portion of the polynucleotide of the second organism.
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43. The method of claim 42, wherein the step of subjecting comprises jointly subjecting the first, second, and third amplicons to temperature gradient electrophoresis along a common electrophoresis lane.
- 15 44. The method of claim 43, wherein the electrophoresis lane is a capillary.
45. The method of claim 29, comprising determining the presence of a difference between a sequence of the first portion of the polynucleotide of the first organism and a sequence of the first portion of the polynucleotide of the second organism based on the electrophoresis data.
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46. The method of claim 15, wherein (i) the step of subjecting the respective first amplicons of the first and second organisms to temperature gradient electrophoresis comprises (a) amplicon injection, wherein at least the first amplicons are introduced to a separation lane, and (b) electrophoresis, wherein at least the first amplicons migrate along the separation lane and, (ii) wherein the step of subjecting a mixture comprising the respective denatured first amplicons of the first and second organisms to annealing is performed prior to electrophoresis.
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47. A method of determining a sequence of a portion of a polynucleotide of a first organism, comprising:
- 30 amplifying: (a) a plurality of sub-portions of a polynucleotide of a first

- organism to prepare amplicons corresponding to the sub-portions of the polynucleotide of the first organism and (b) a plurality of sub-portions of a polynucleotide of a second organism to prepare amplicons corresponding to the sub-portions of the polynucleotide of the second organism, the sub-portions of the second organism each having a known sequence;
- 5 forming a plurality of duplexes comprising an amplicon of the first organism and an at least partially complementary amplicon of the second organism;
- subjecting the duplexes to temperature gradient electrophoresis to obtain, for respective duplexes, electrophoresis data indicative of the presence of a mismatch between the amplicon of the first organism and the at least partially complementary amplicon of the second organism;
- 10 determining duplexes having a mismatch;
- for duplexes determined to have a mismatch, determining the identity of the mismatch between the amplicon of the first organism and the at least partially complementary amplicon of the second organism based on the known sequences of the corresponding sub-portion of the second organism; and
- 15 determining the sequence of at least a portion of the polynucleotide of the first organism based on (a) the known sequences of sub-portions of the second organism determined from the electrophoresis data not to have a mismatch with sub-portions of the first organism and (b) the identity of mismatches between amplicons of the first and second organisms.
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48. A method of determining whether a sequence of a first portion of a polynucleotide of a first organism and a sequence of a first portion of a polynucleotide of a second organism comprise a difference, the method comprising:
- 25 amplifying at least a first portion of a polynucleotide of a first organism to prepare first amplicons of the first organism;
- amplifying at least a first portion of a polynucleotide of a second organism to prepare first amplicons of the second organism;
- 30 denaturing the first amplicons of the first organism;
- denaturing the first amplicons of the second organism;
- subjecting a mixture comprising the respective denatured first

amplicons of the first and second organisms to annealing; and
subjecting the respective first amplicons of the first and second
organisms to temperature gradient electrophoresis to obtain first
electrophoresis data indicative of the presence of a difference between (a) a
sequence of the first portion of the polynucleotide of the first organism and (b)
a sequence of the first portion of the polynucleotide of the second organism.

- 5 49. A method of determining whether a sequence of a first portion of a
polynucleotide of a first organism and a sequence of a first portion of a
polynucleotide of a second organism comprise a difference, the method
comprising:
- 10 combining a polynucleotide of a first organism and a
polynucleotide of a second organism, the polynucleotide of the first organism
comprising a first portion and the polynucleotide of the second organism
comprising a first portion;
- 15 amplifying at least the first portion of the polynucleotide of the
first organism and the first portion of the polynucleotide of the second
organism to prepare first amplicons comprising the amplified first portions;
- 20 denaturing the first amplicons;
 annealing the first amplicons; and
 subjecting the first amplicons to temperature gradient
electrophoresis to obtain first electrophoresis data indicative of the presence of
a difference between a sequence of the first portion of the polynucleotide of
the first organism and a sequence of the first portion of the polynucleotide of
the second organism.
- 25 50. The method of claim 49, wherein the first organism is a first mammal.
51. The method of claim 50 wherein the first organism is a human.
52. The method of claim 51, wherein the second organism is a second,
different mammal.
53. The method of claim 52, wherein the second organism is a second,
different human.
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54. The method of claim 53, wherein the polynucleotide of the first organism comprises genomic DNA of the first organism.
55. The method of claim 54, wherein the polynucleotide of the second organism comprises genomic DNA of the second organism.
- 5 56. A method of determining a sequence of at least a portion of a genome of a first organism, comprising:
10 providing reference DNA obtained from a genome of a reference organism;
providing PCR primers suitable for amplifying the reference DNA;
using the PCR primers to amplify the reference DNA in the presence of the portion of the genome of the first organism to thereby obtain amplicons;
subjecting the amplicons to temperature gradient electrophoresis (TGE) to obtain electrophoresis data;
15 identifying at least one amplicon indicative of a variance between the reference DNA and the portion of the genome of the first organism based on the electrophoresis data;
sequencing the at least one amplicon indicative of a variance to determine the sequence of the portion of the genome of the first organism.
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